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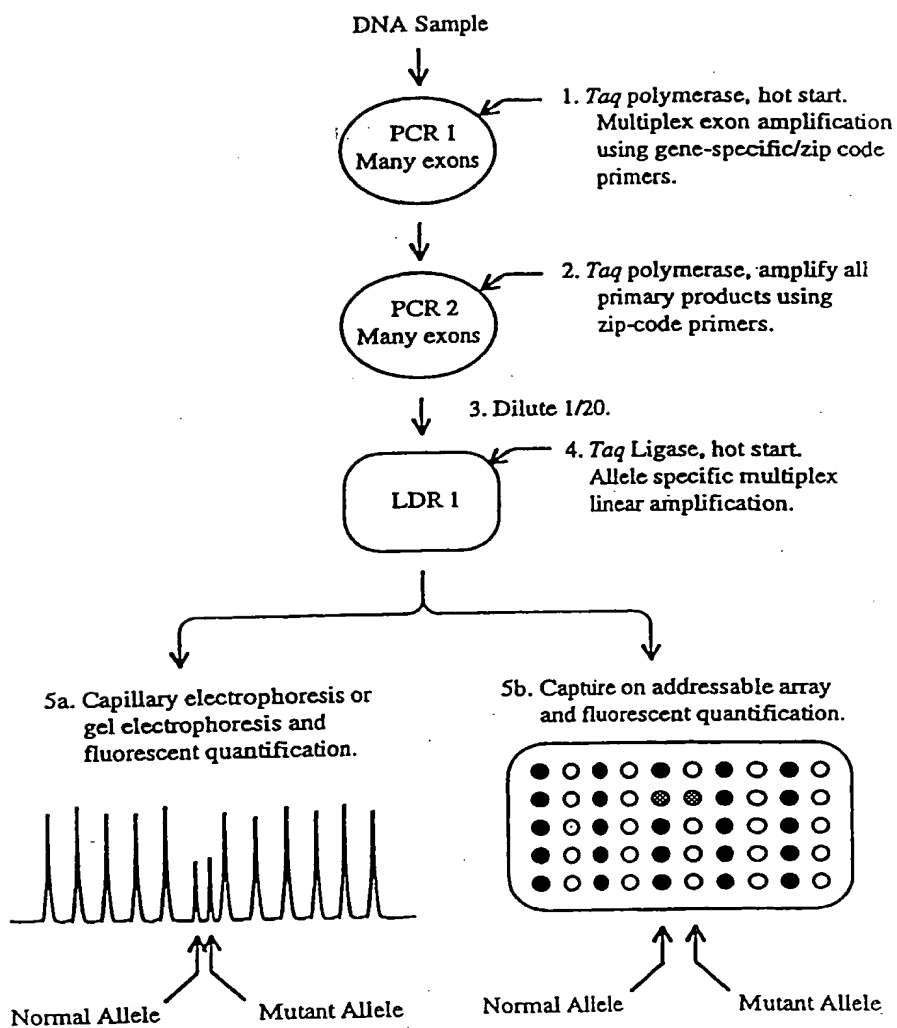


FIG. 1

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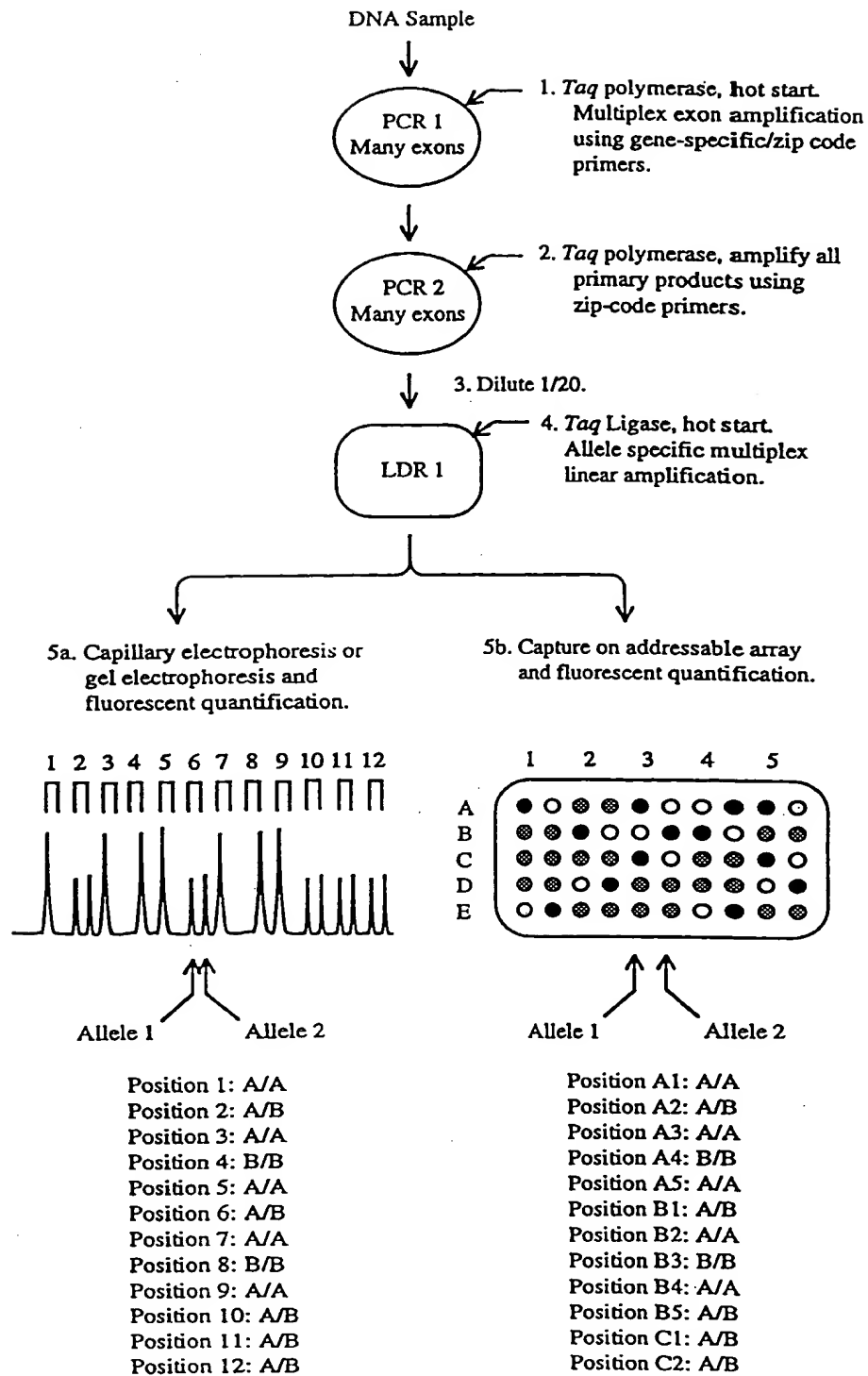
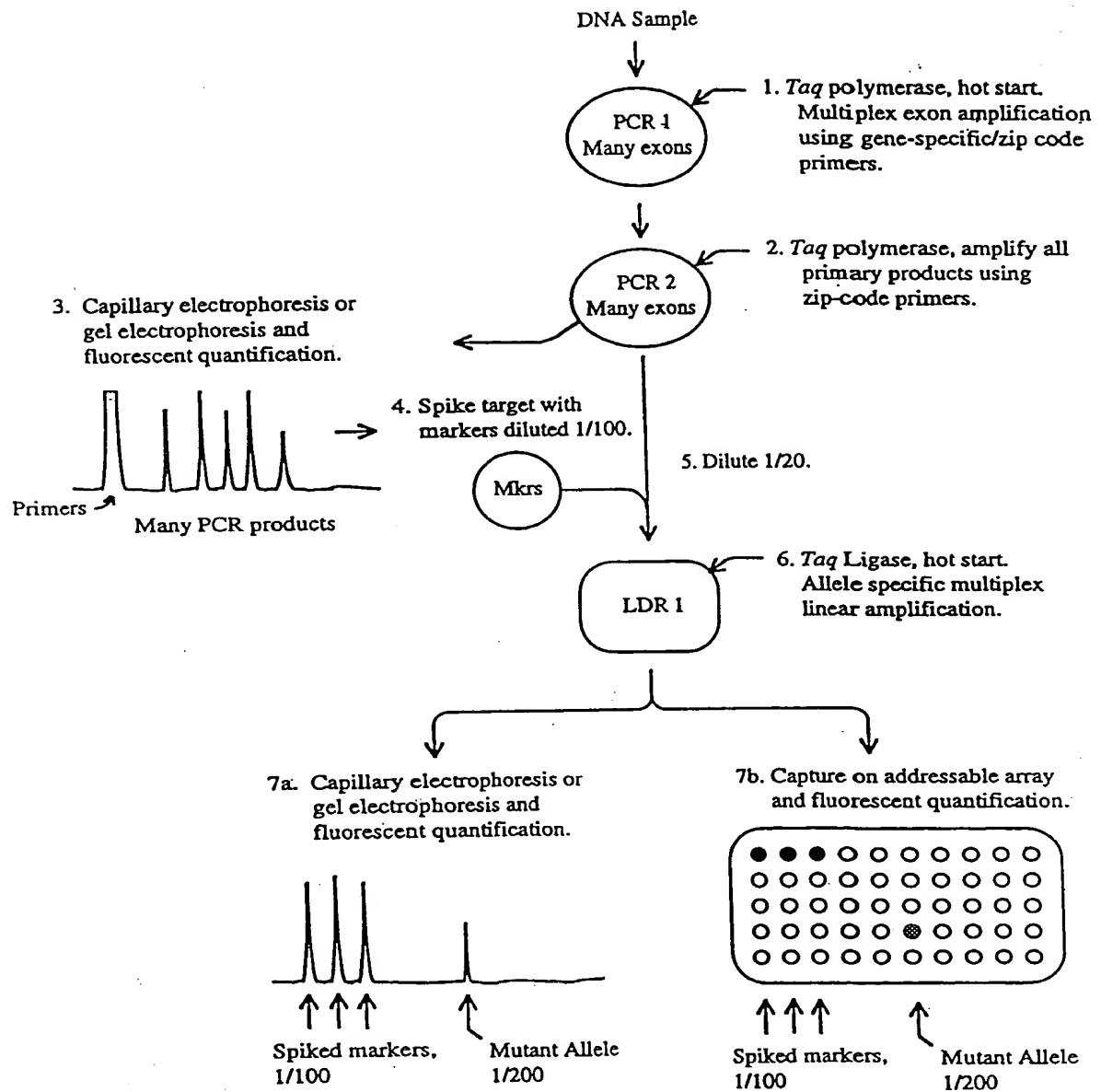
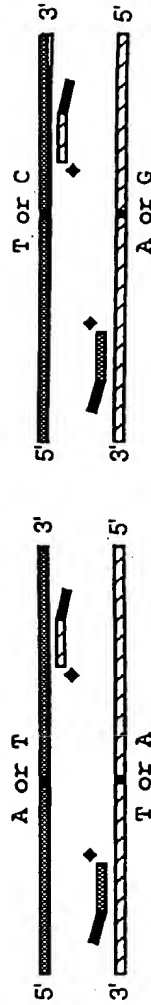


FIG. 2

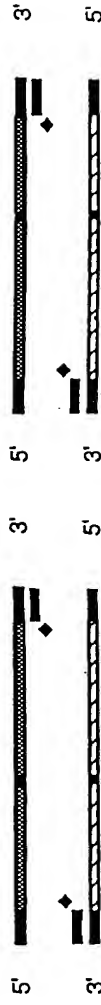
**FIG. 3**

PCR/PCR/LDR

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and Taq polymerase. ♦

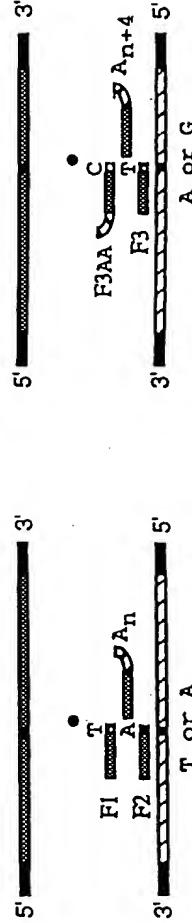


2. PCR amplify all primary products using zip code primers, dNTPs and Taq polymerase.

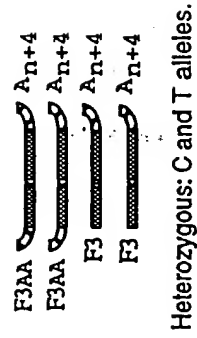


3. Perform LDR using allele-specific LDR primers and thermostable ligase. ●

Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



4. Separate fluorescent products on a DNA sequencer and quantify each allele.



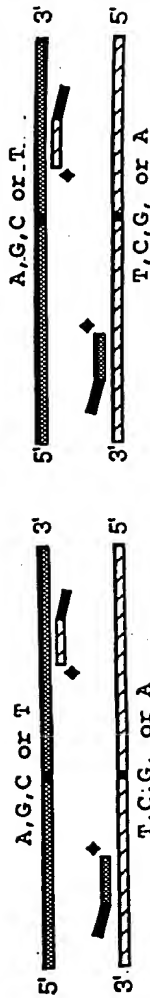
Homozygous: T allele only.

Heterozygous: C and T alleles.

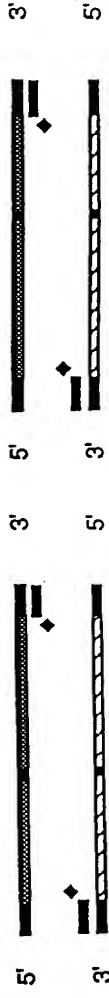
FIG. 4

PCR/PCR/LDR

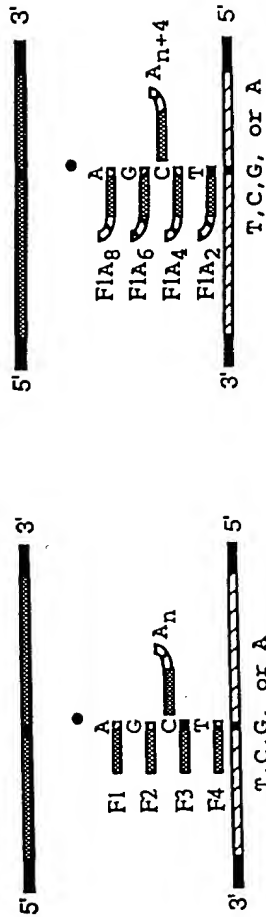
1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and Taq polymerase. ♦



2. PCR amplify all primary products using zip code primers, dNTPs and Taq polymerase.



3. Perform LDR using allele-specific LDR primers and thermostable ligase. ●
Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



4. Separate fluorescent products on a DNA sequencer and quantify each allele.



FIG. 5

PCR/PCR/LDR : Nearby alleles

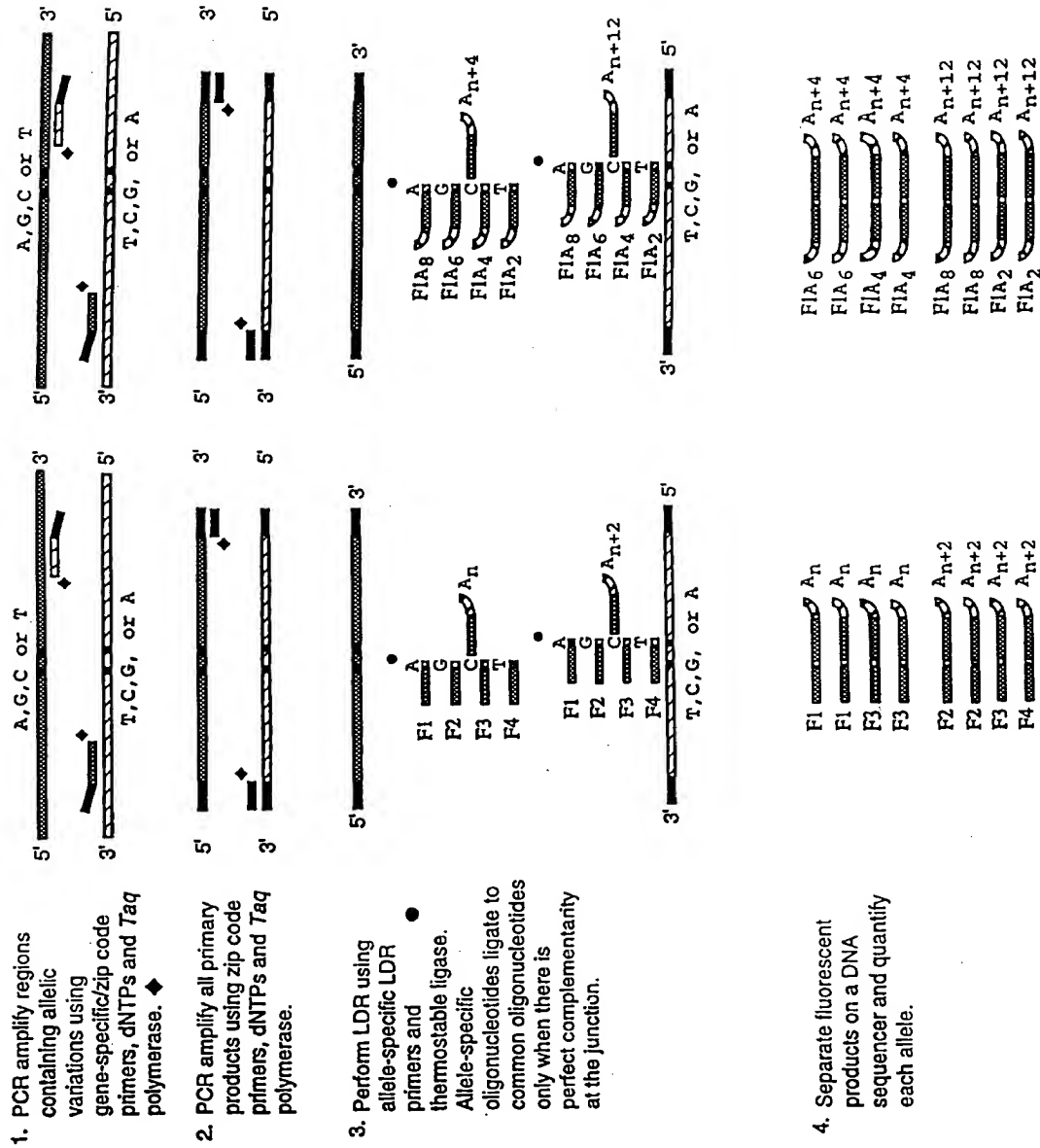


FIG. 6

1st Position.
Heterozygous: A and C alleles.
2nd Position.
Heterozygous: G, C and T alleles.

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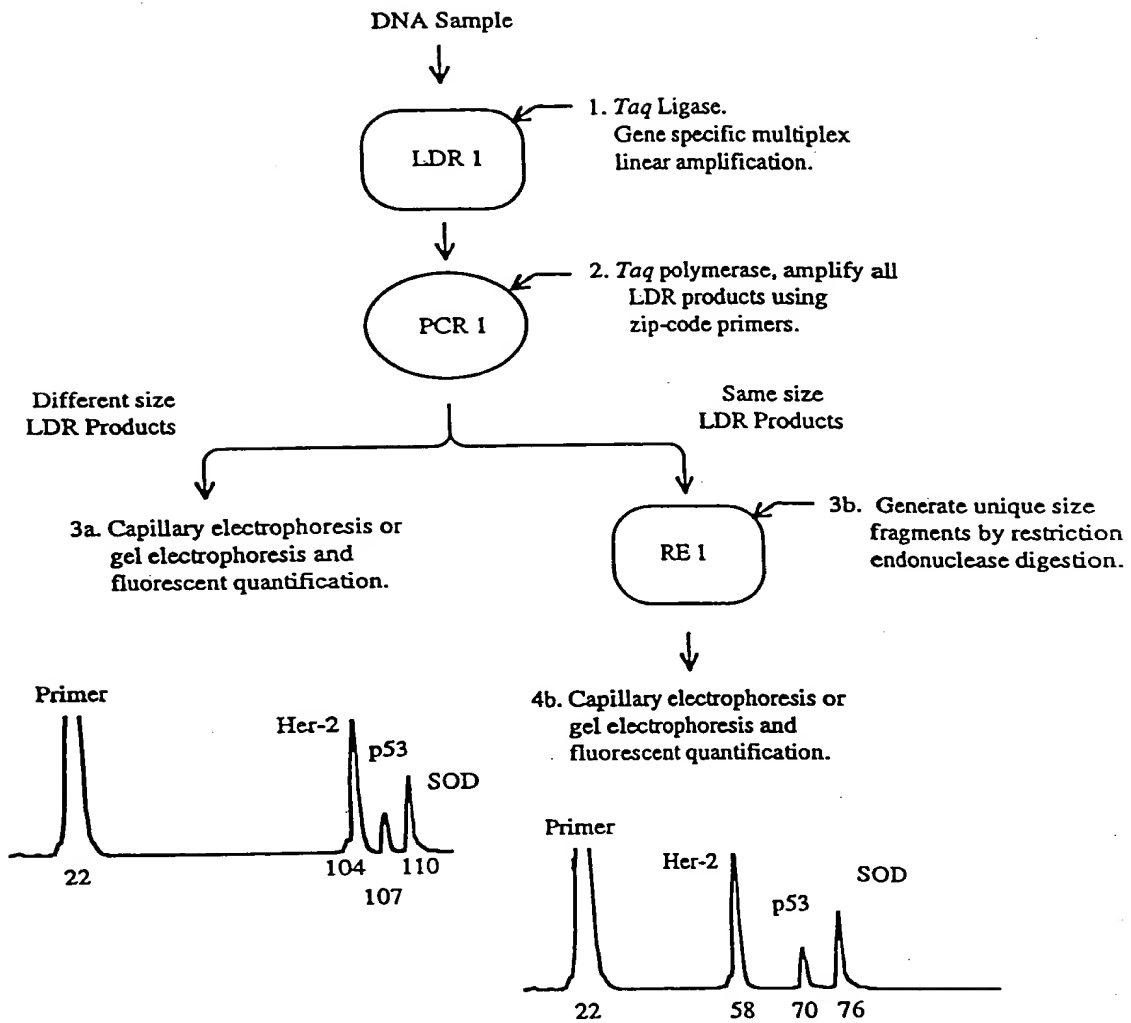


FIG. 8

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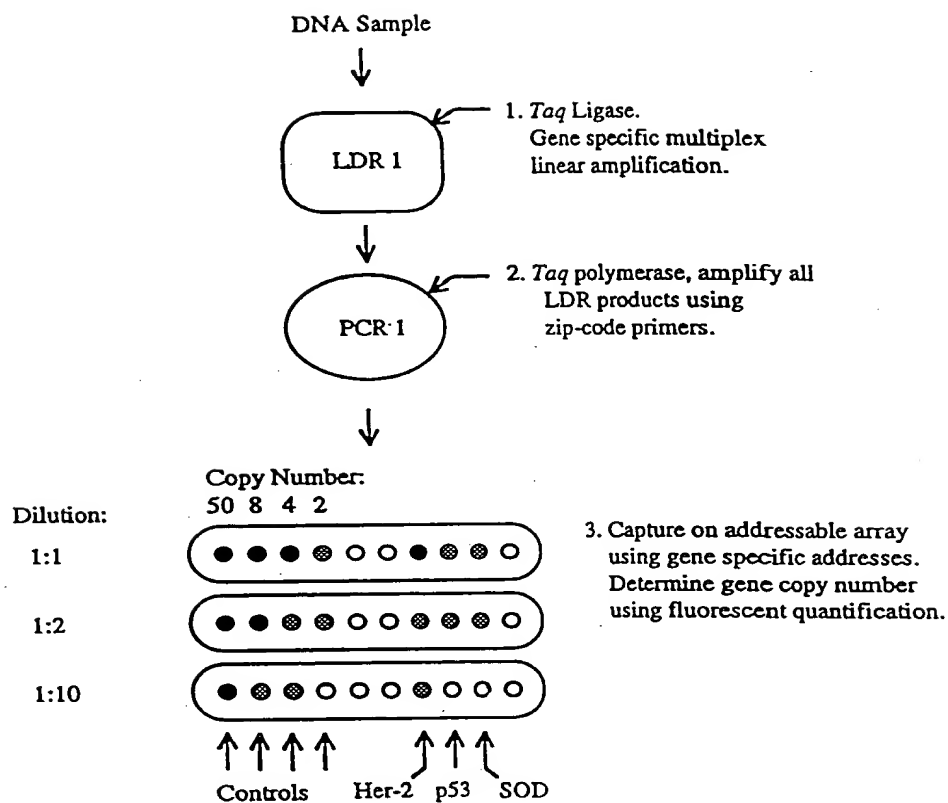


FIG. 9

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LDR / PCR: Multiplex detection of gene amplifications and deletions

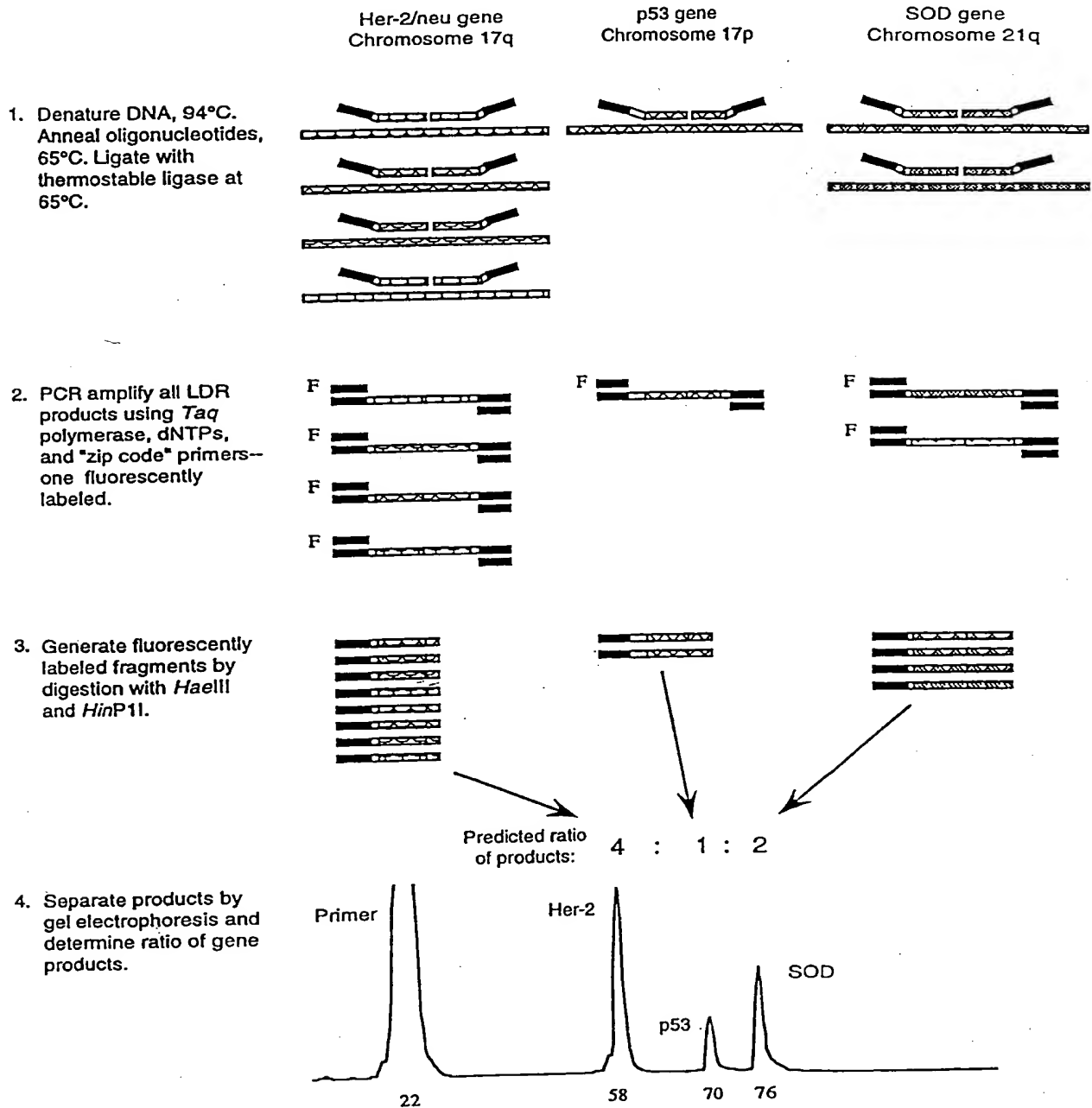


FIG. 10

Allele specific LDR / PCR Problem

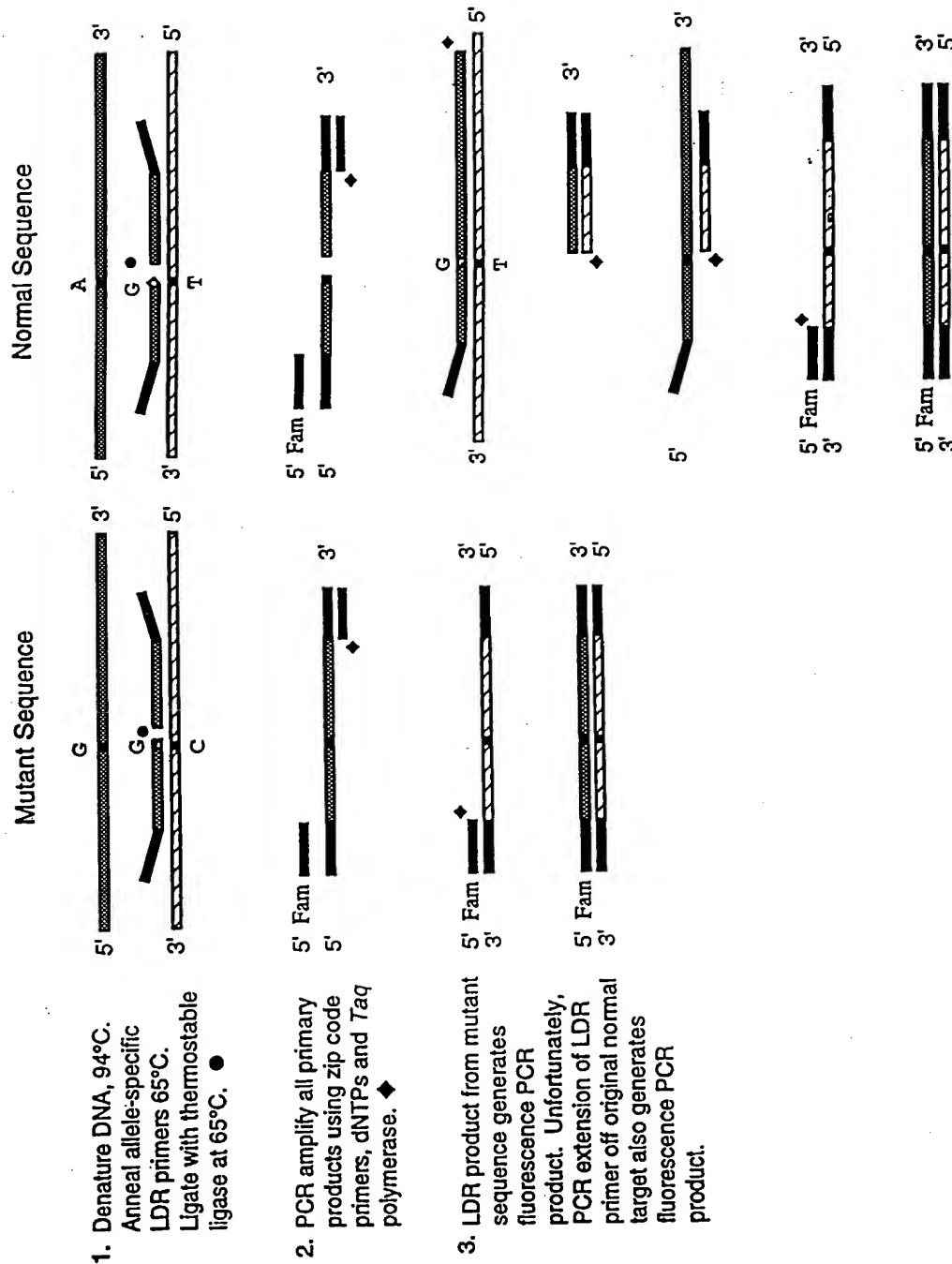


FIG. 11

Solution to allele specific LDR / PCR problem

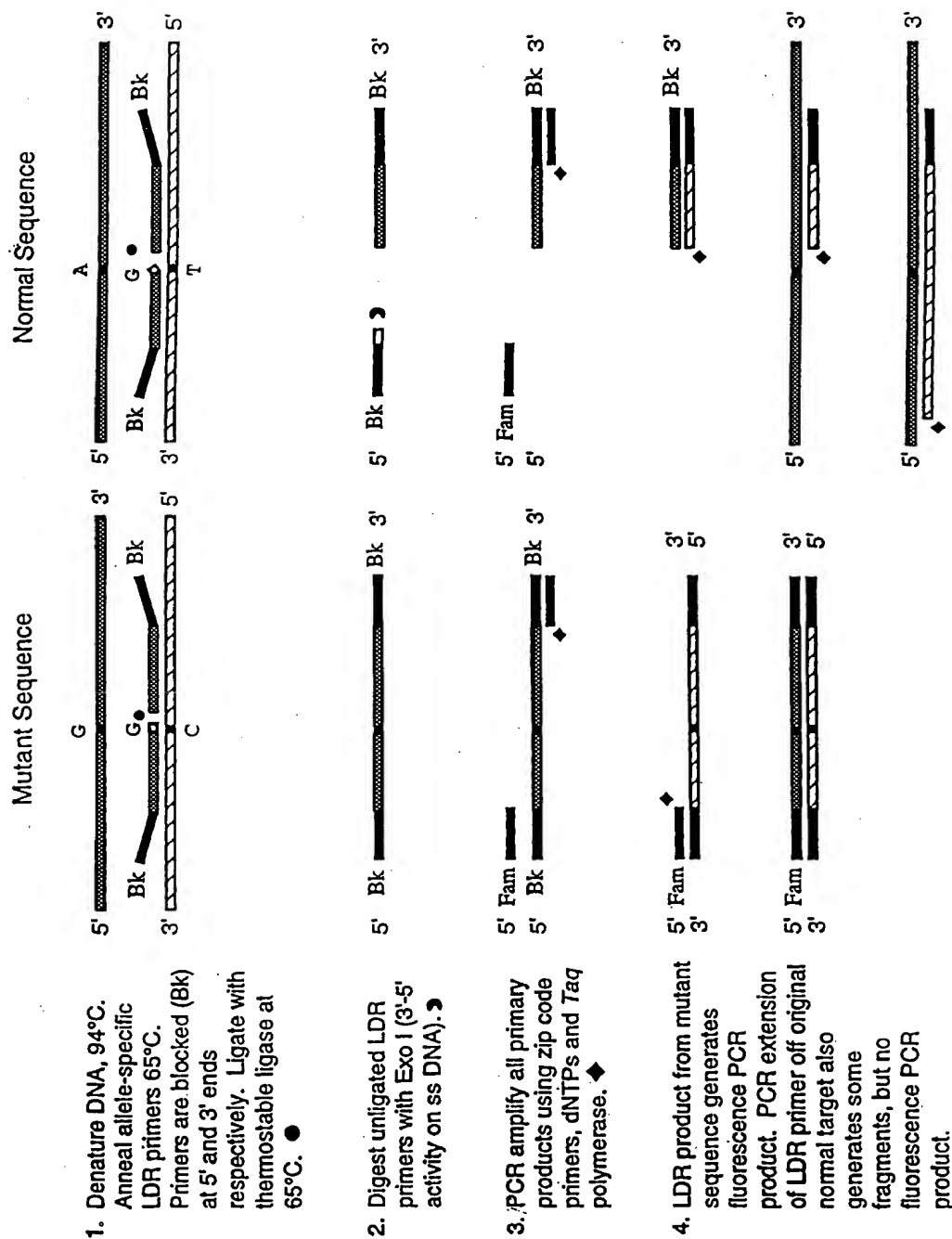


FIG. 12

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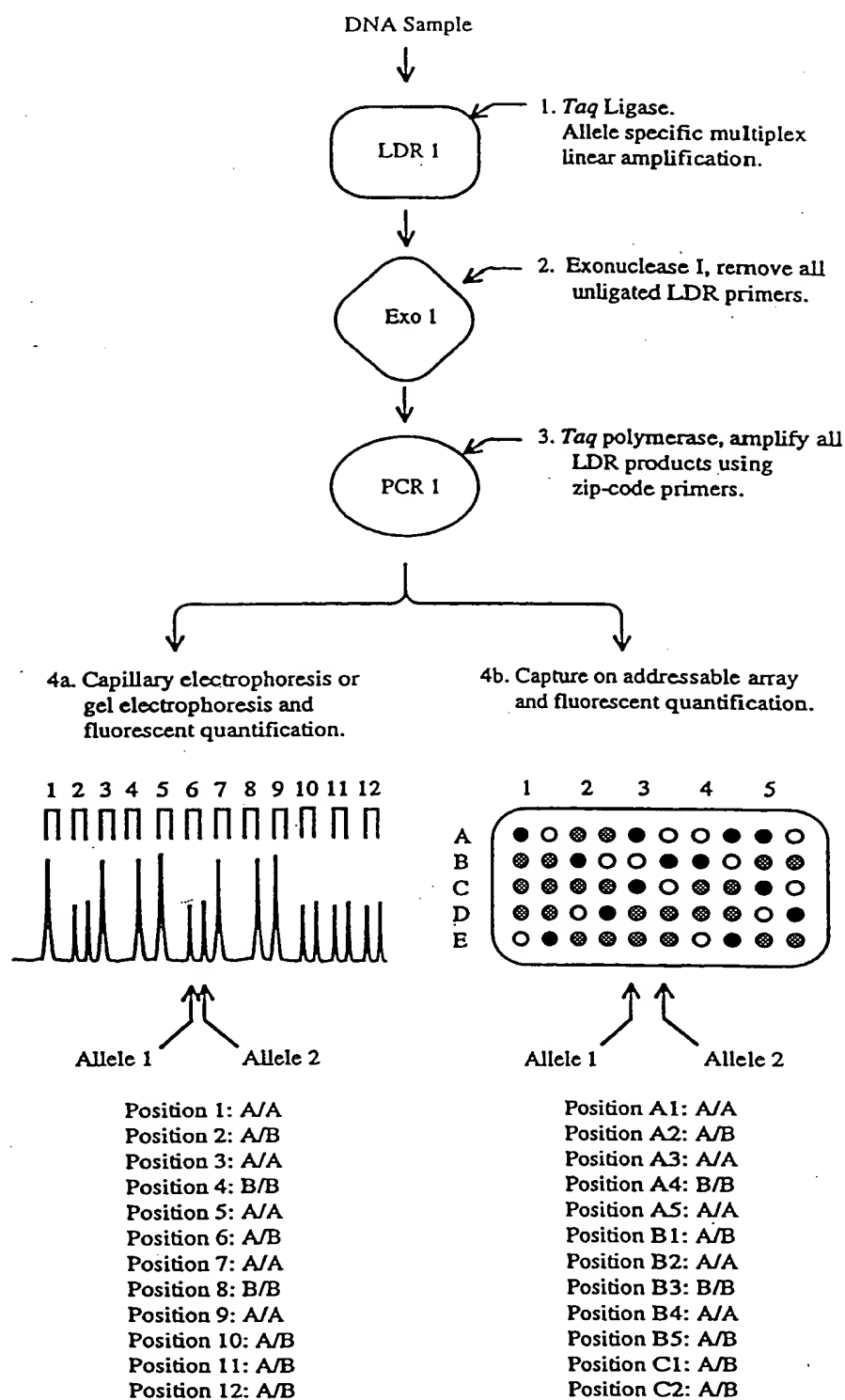


FIG. 13

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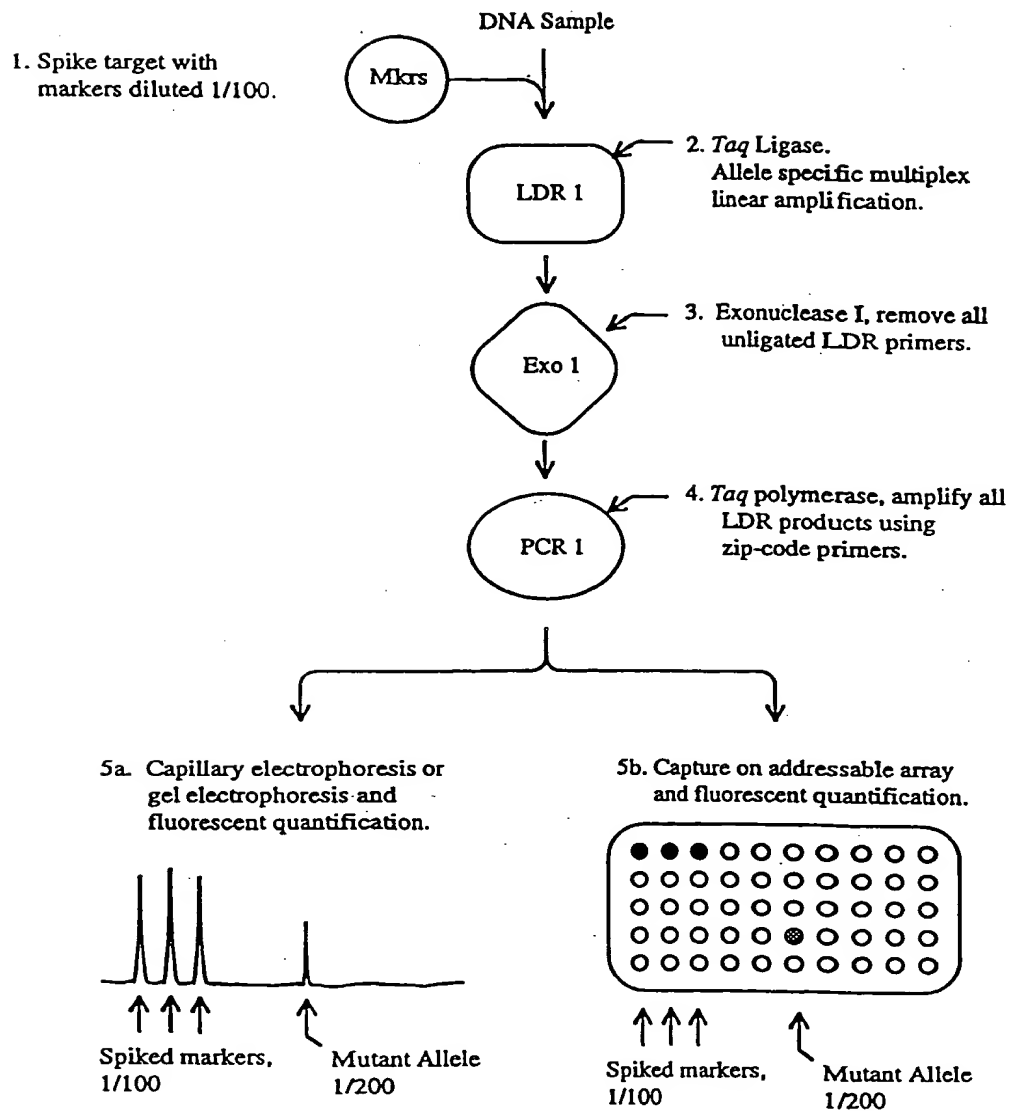


FIG. 14

Allele specific LDR / PCR for mutations or polymorphisms

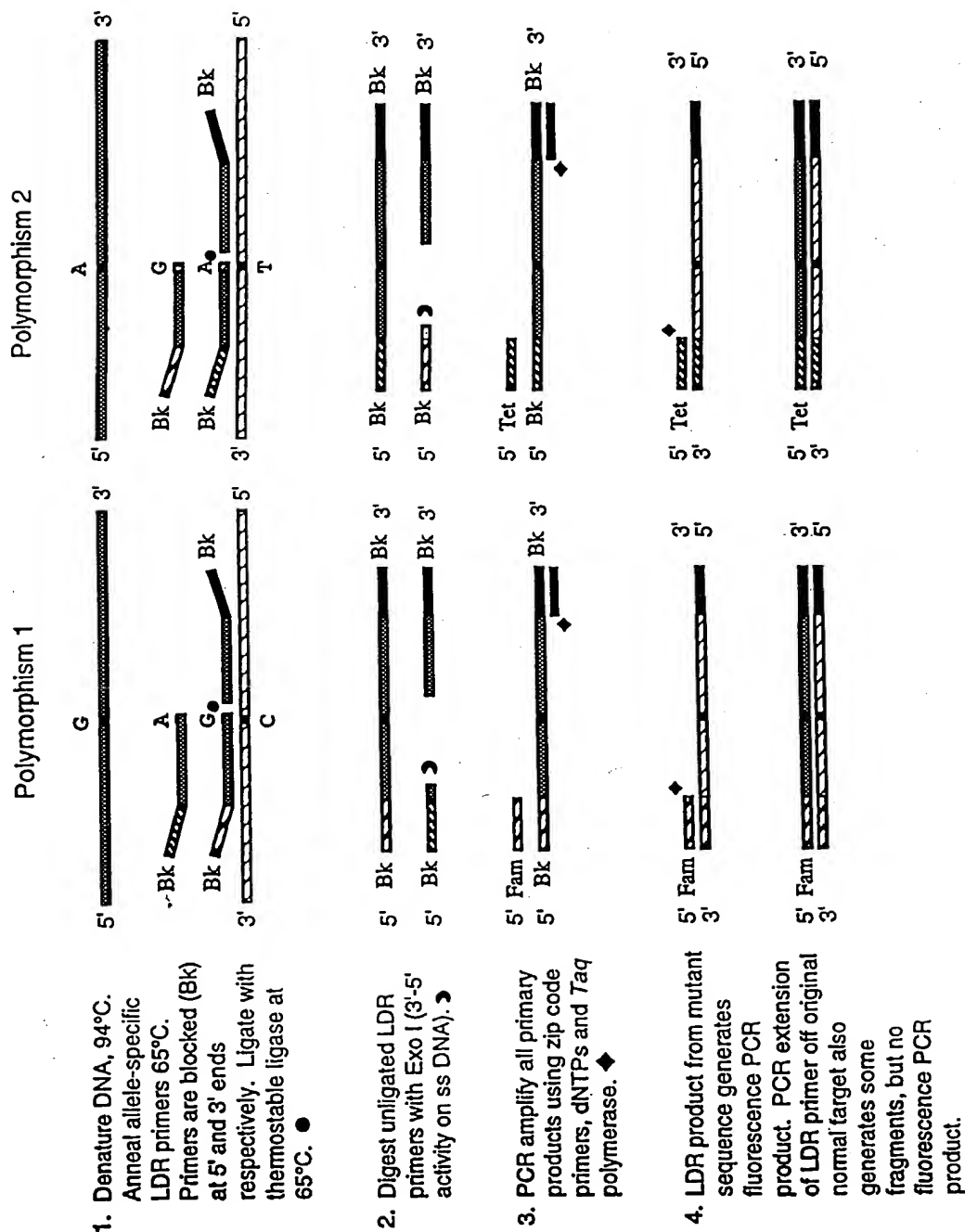


FIG. 15

LDR / PCR of mononucleotide repeats using exonuclease selection

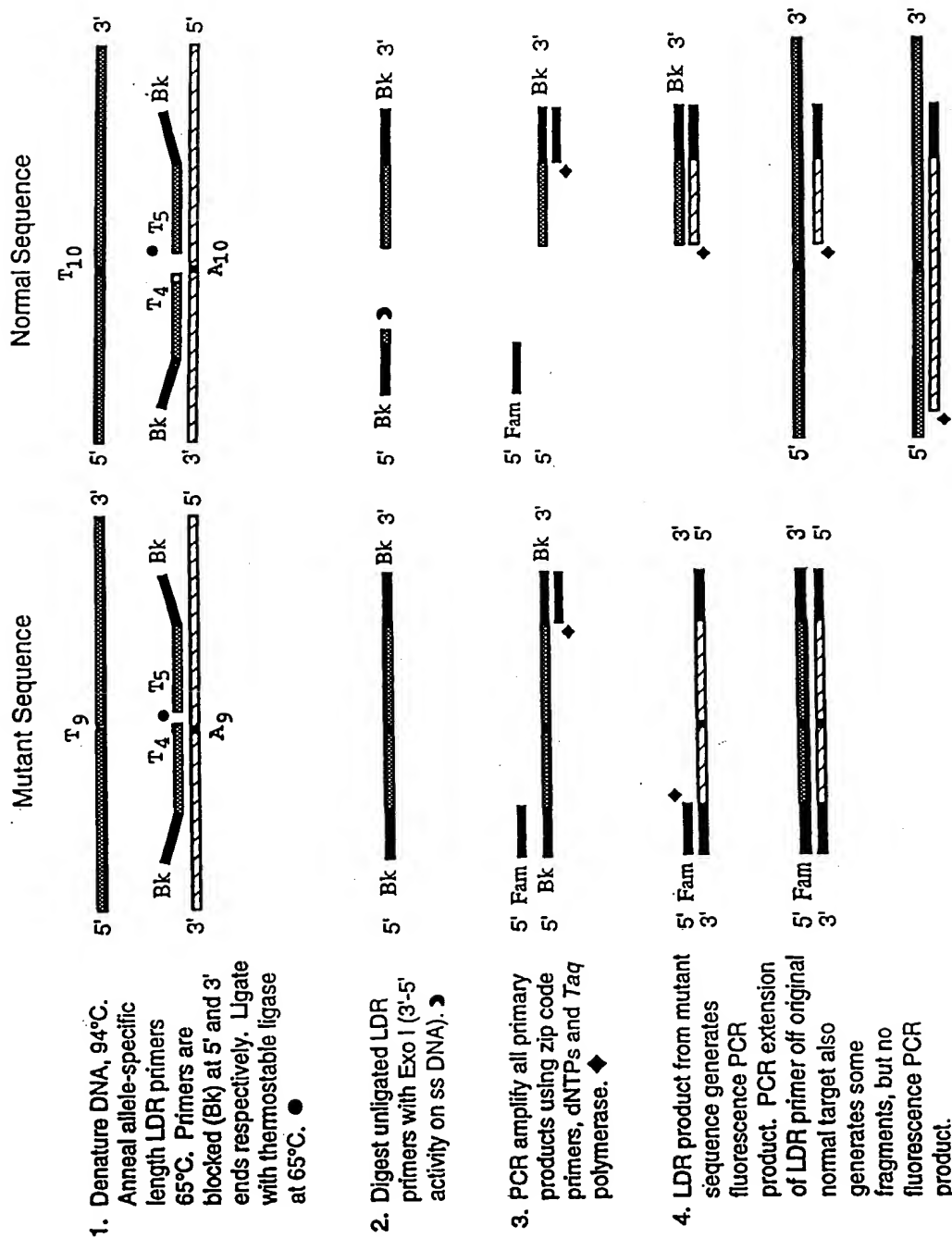


FIG. 16

LDR / PCR of mononucleotide repeat polymorphisms using exonuclease selection

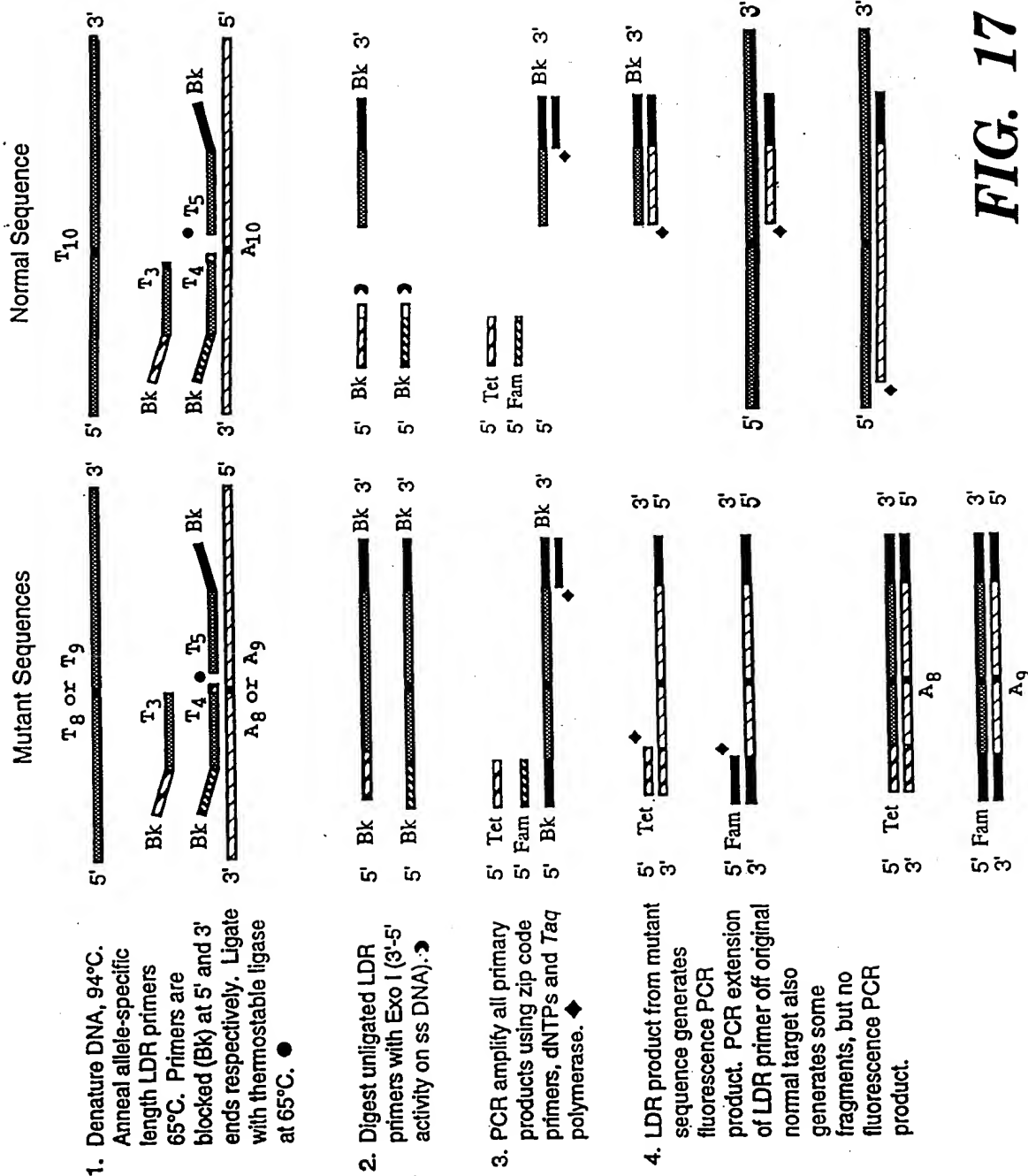


FIG. 17

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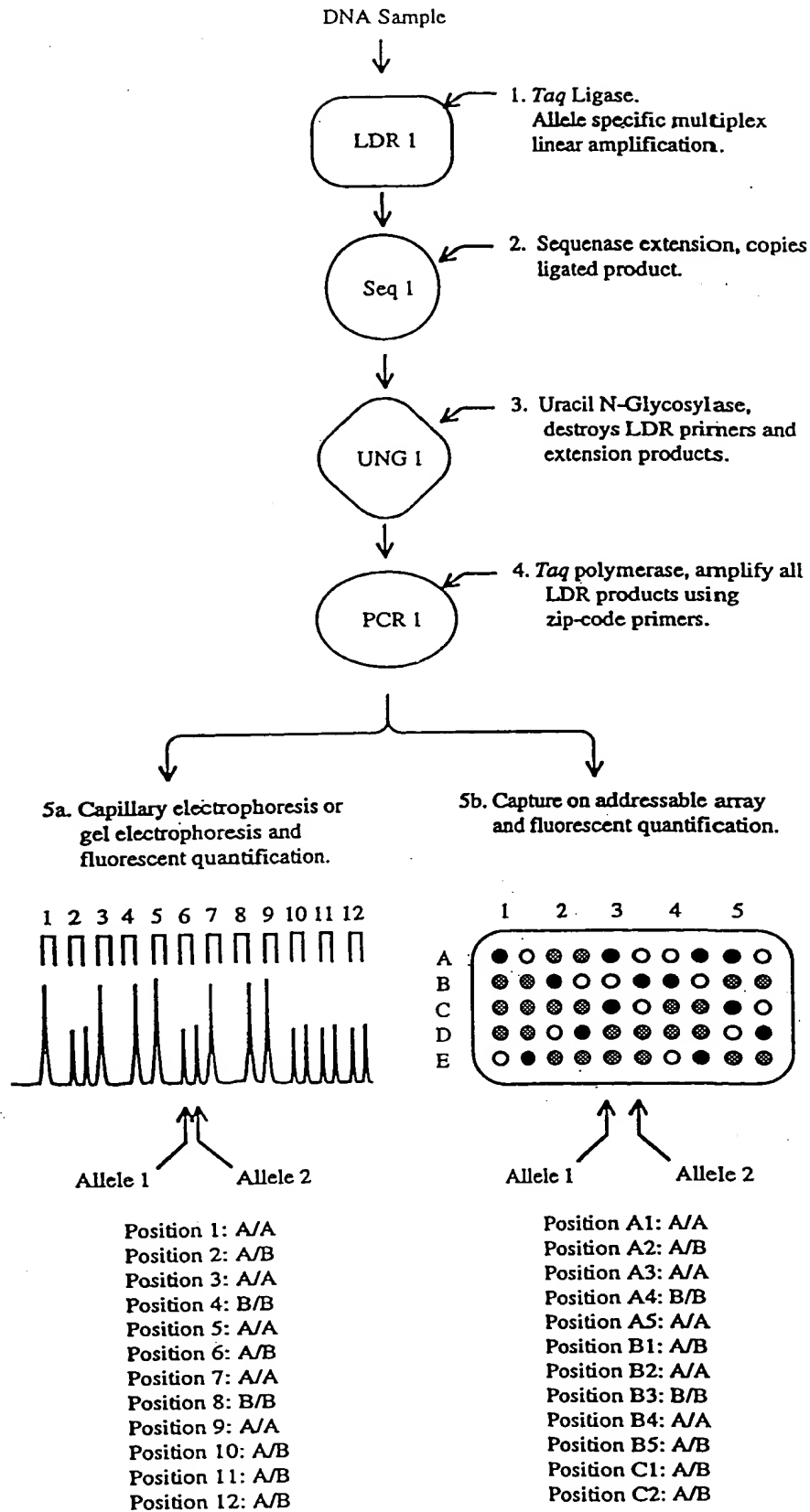


FIG. 18

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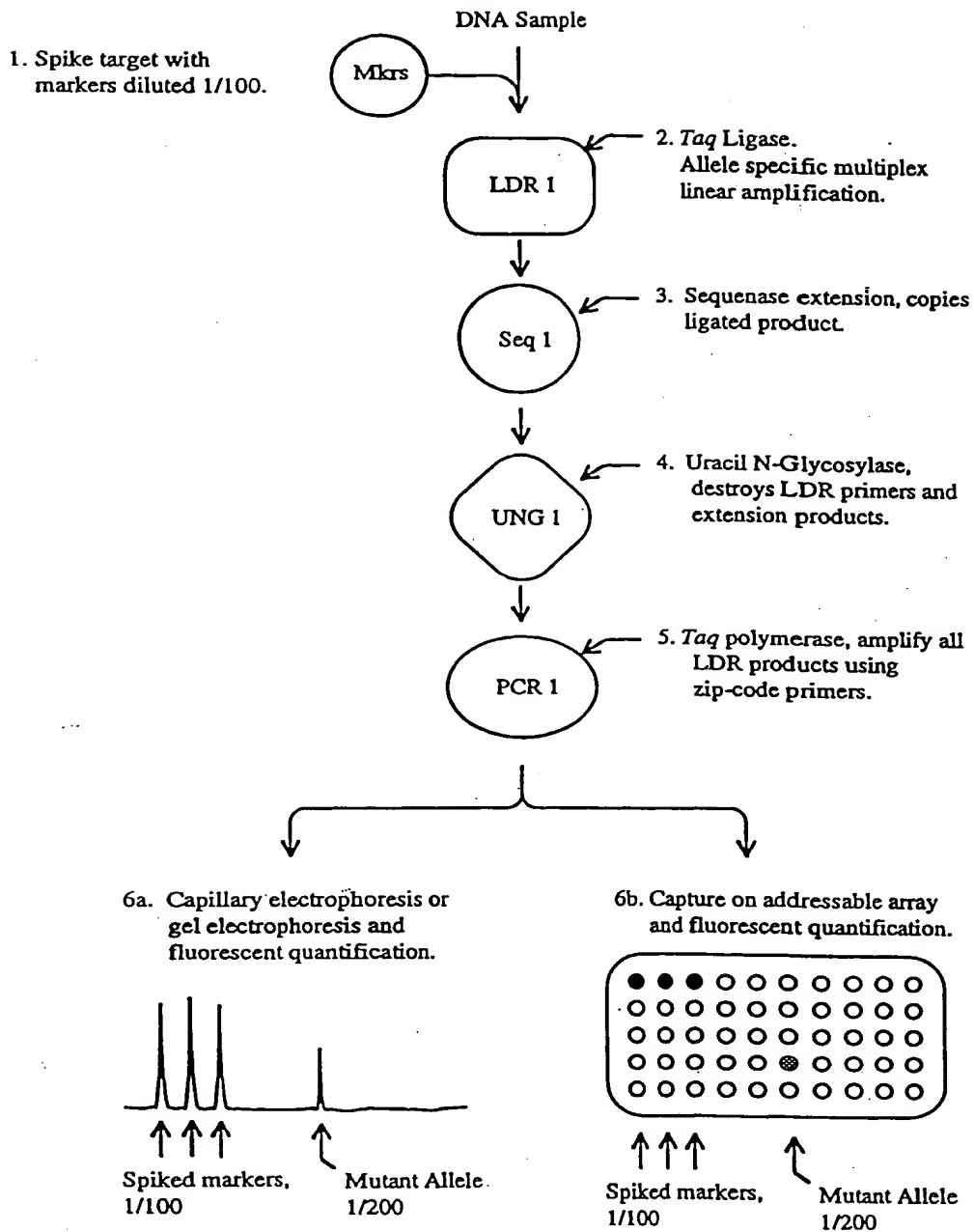


FIG. 19

LDR / PCR of mononucleotide repeats using Uracil N-glycosylase selection

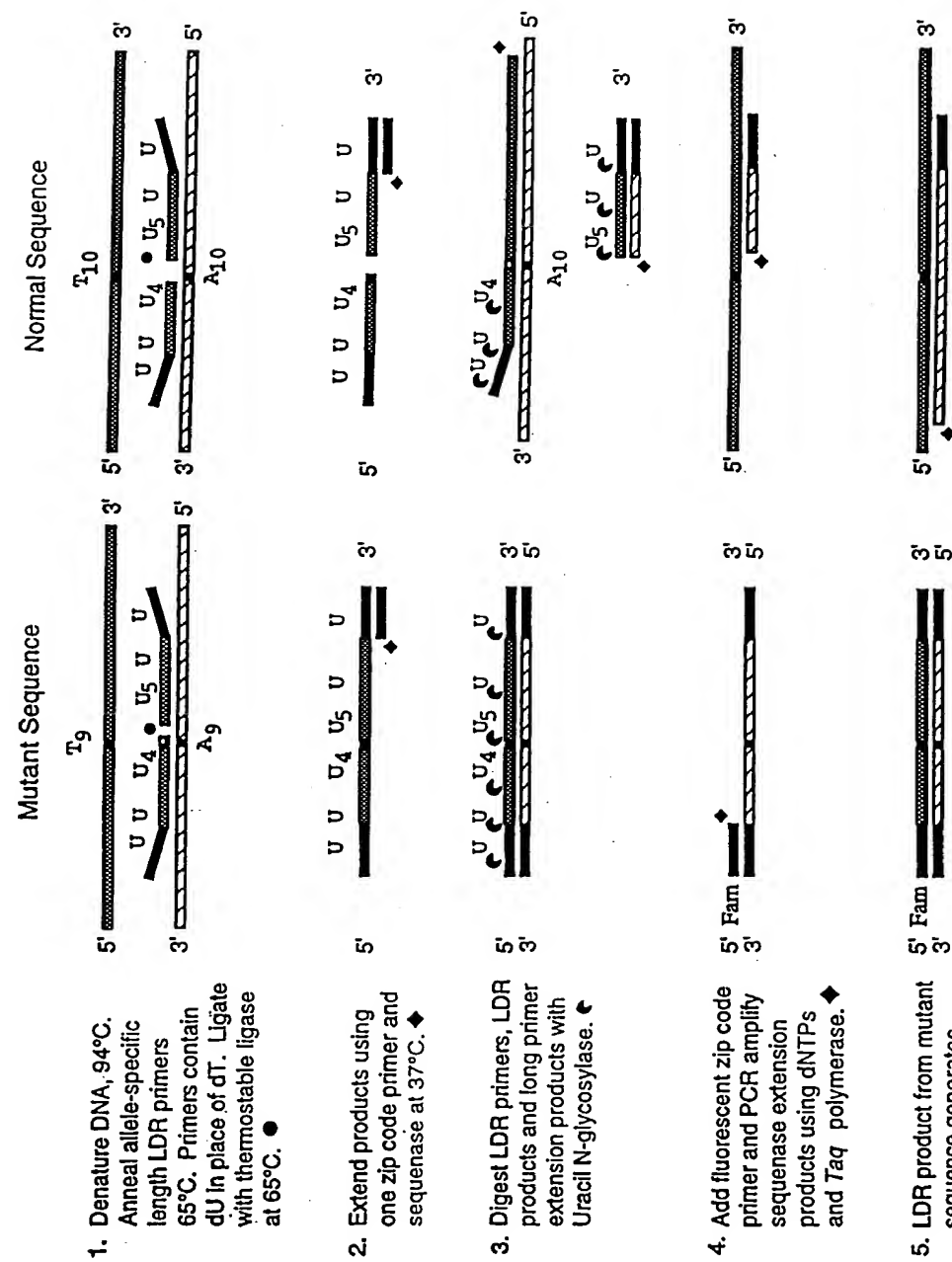


FIG. 20

LDR / PCR of mononucleotide repeat polymorphisms using Uracil N-glycosylase selection

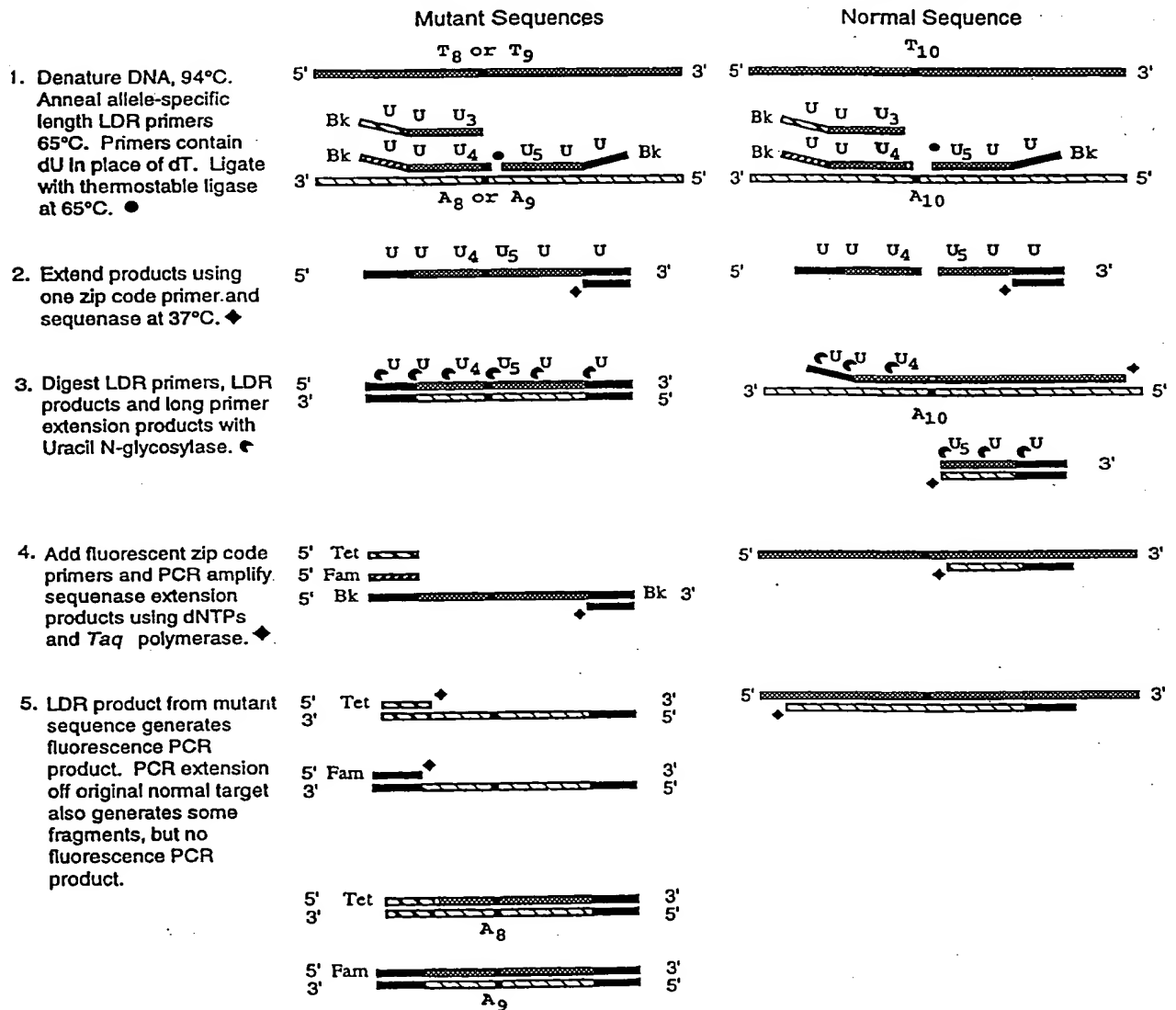
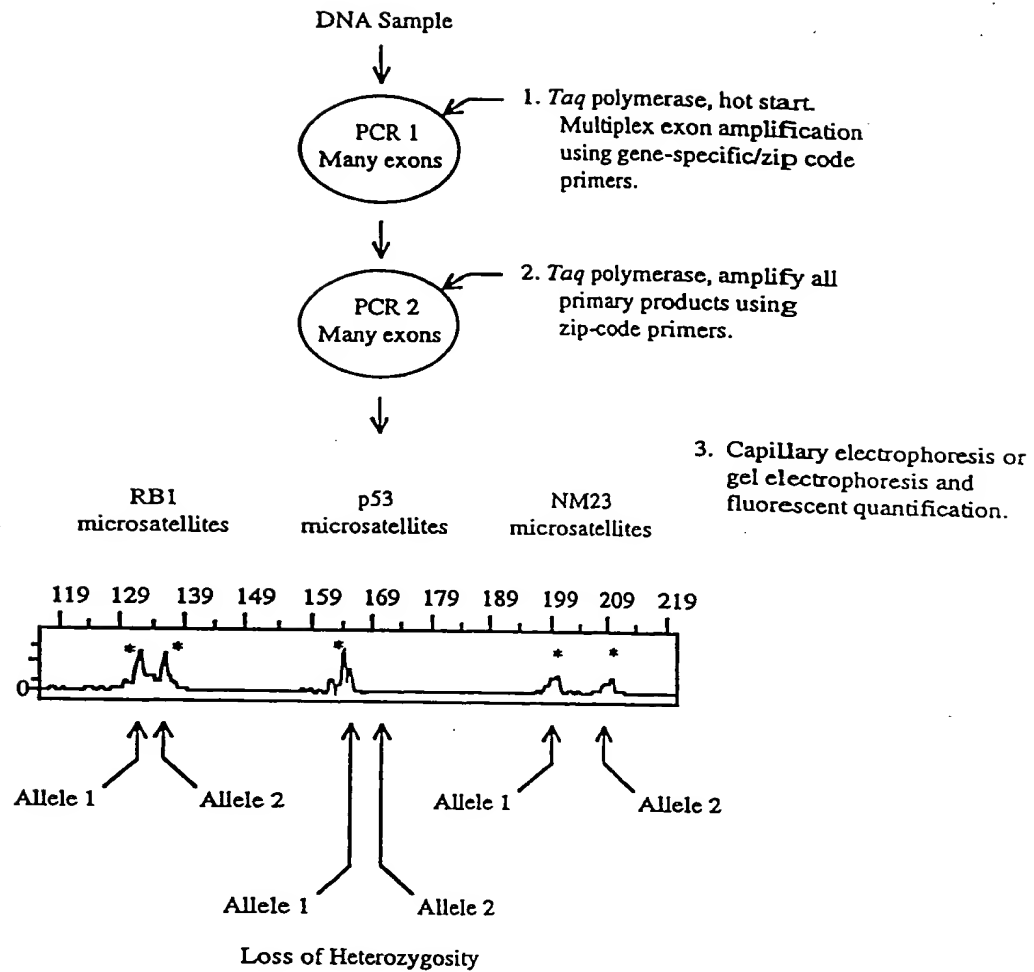
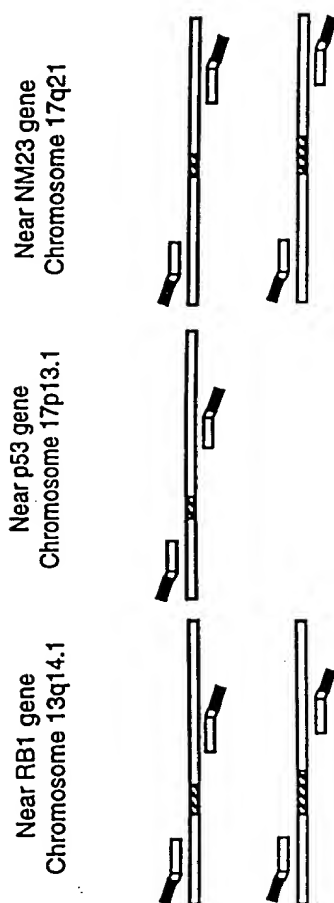


FIG. 21

**FIG. 22**

PCR / PCR : Multiplex Microsatellite assays

1. Denature DNA, 94°C.
Anneal longer
oligonucleotides, 65°C.
PCR amplify for 10 - 15
cycles.



2. PCR amplify all primary products using *Taq* polymerase, dNTPs, and "zip code" primers--one fluorescently labeled. Separate products by gel electrophoresis and determine loss of heterozygosity at informative loci.

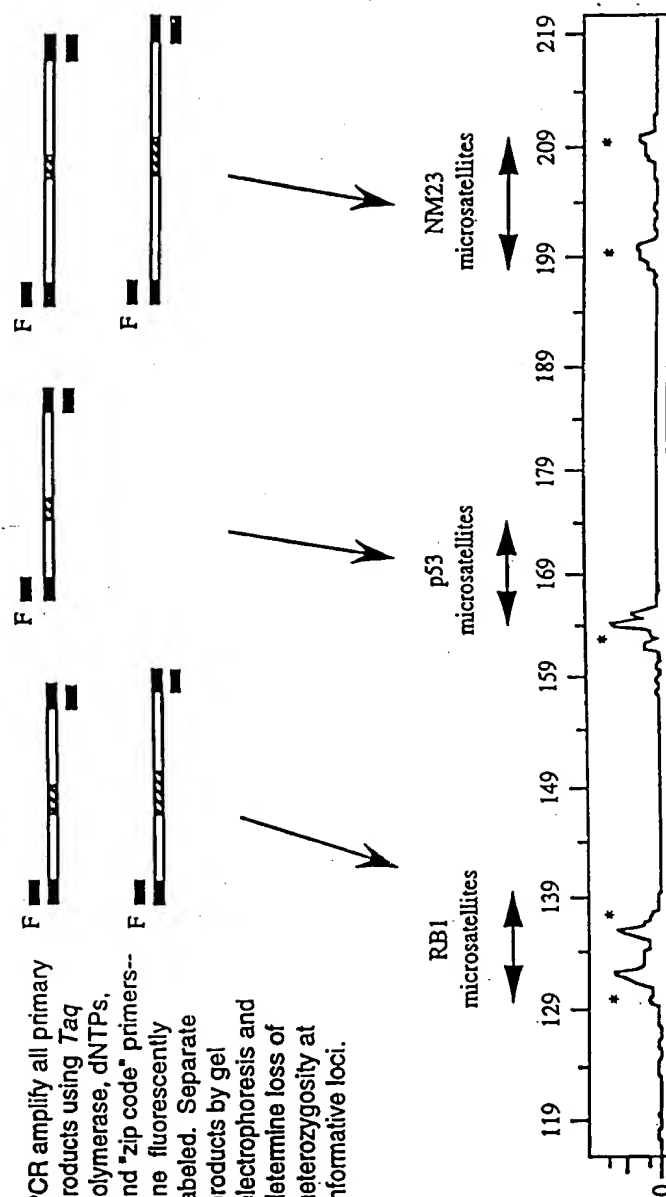


FIG. 23

Primer design for multiplex LDR / PCR

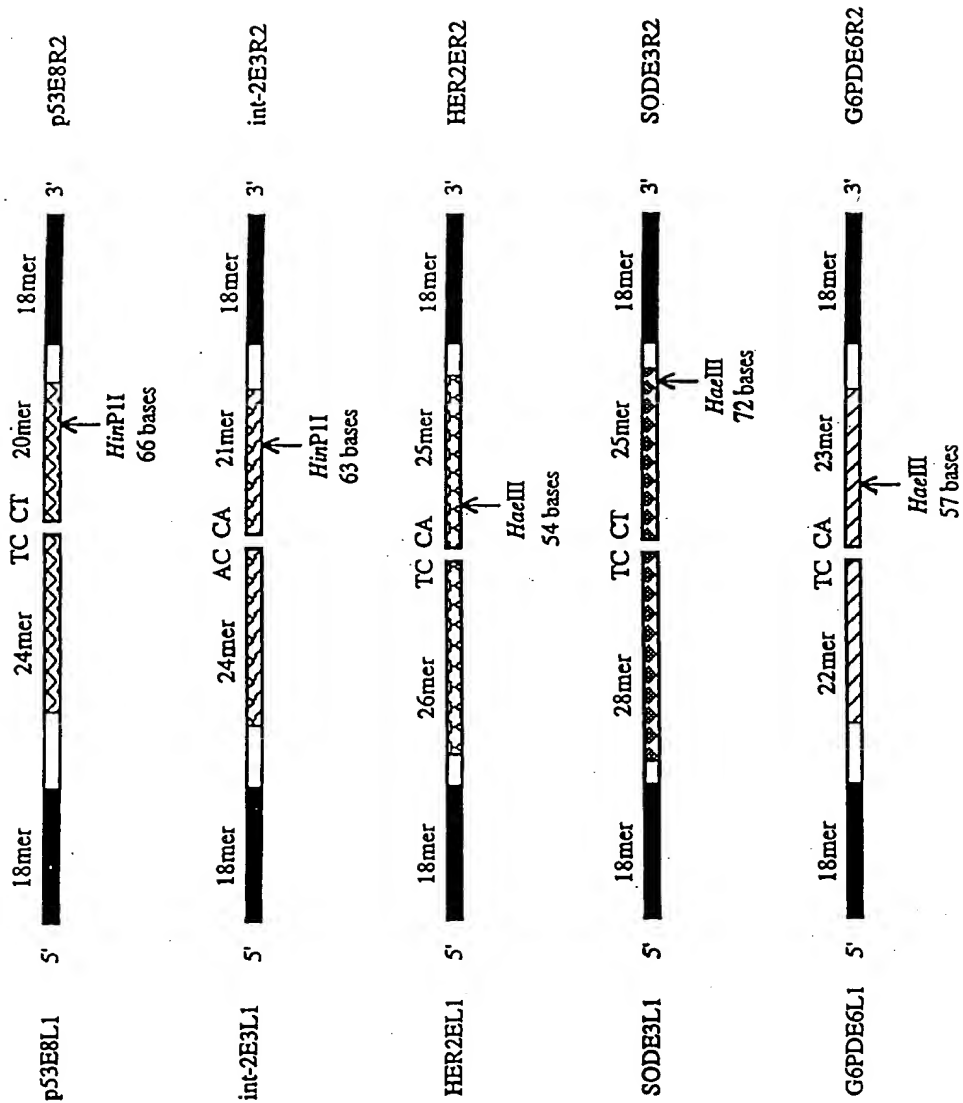


FIG. 24

FIG. 25A

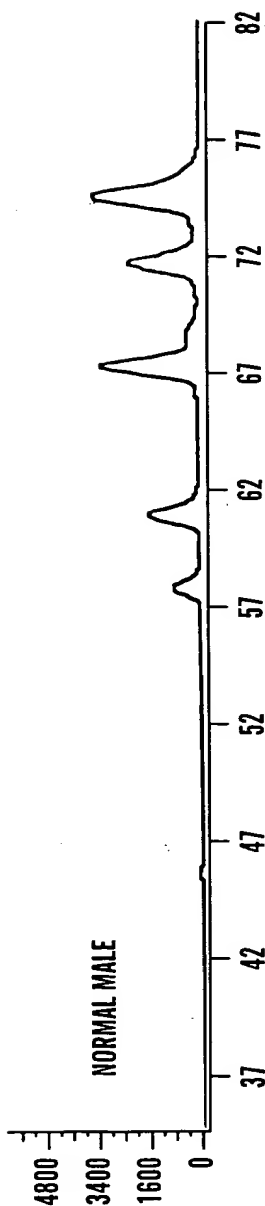


FIG. 25B

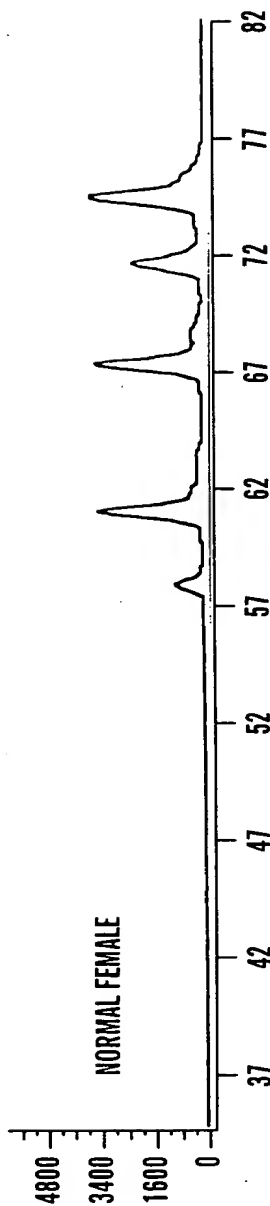


FIG. 25C

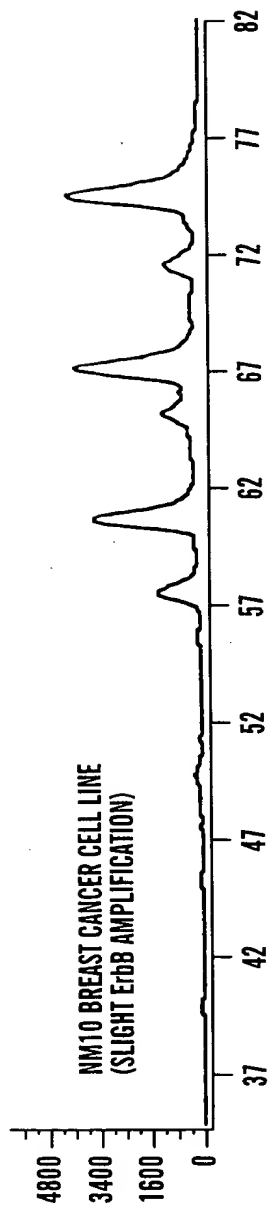


FIG. 25D

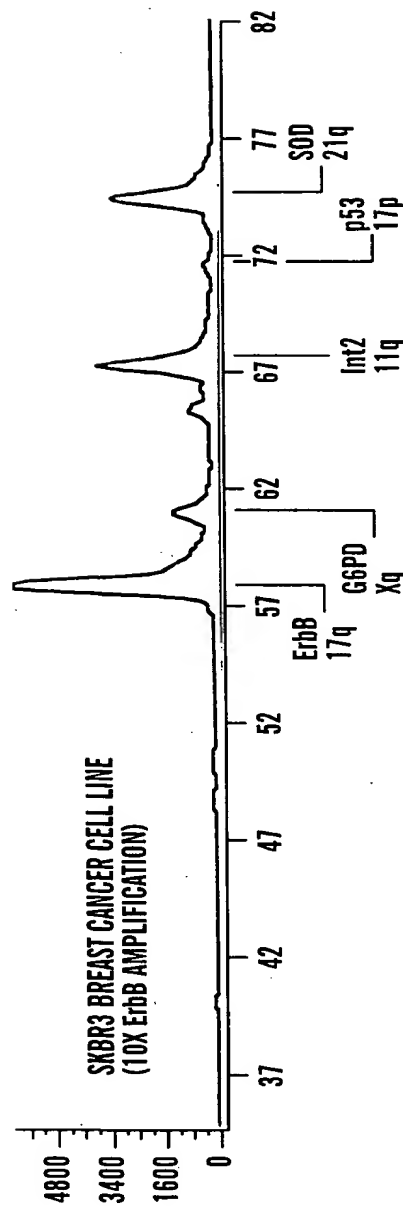


FIG. 26A

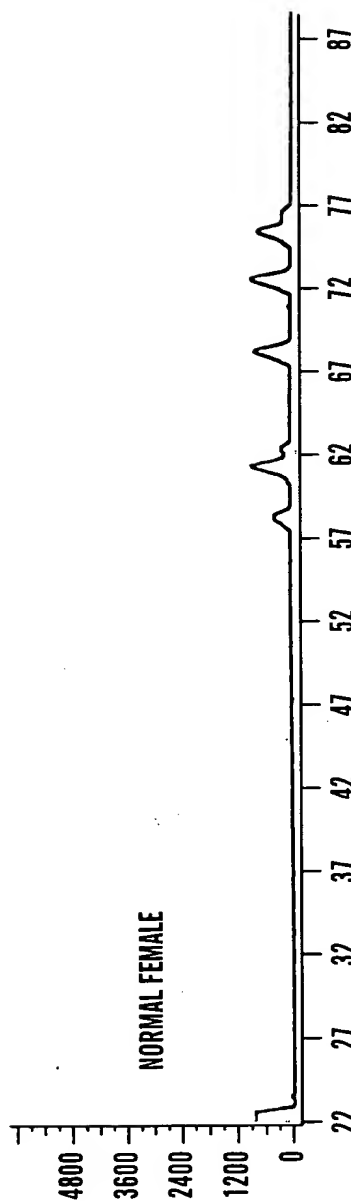


FIG. 26B

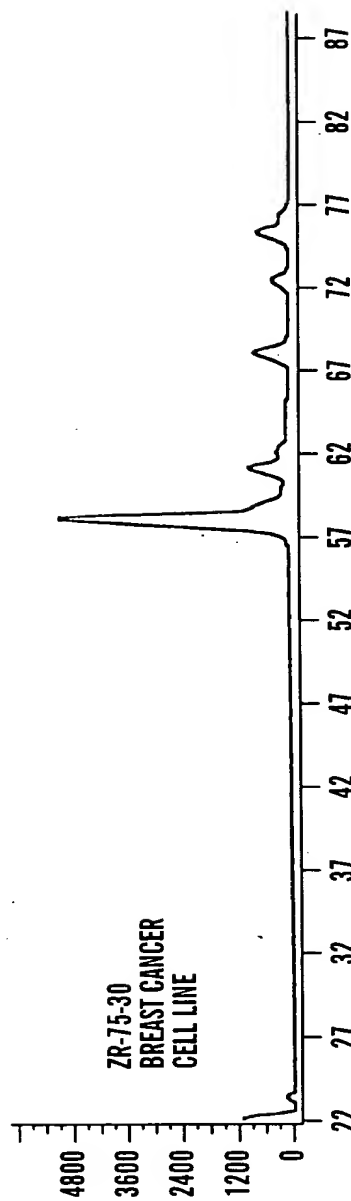


FIG. 26C

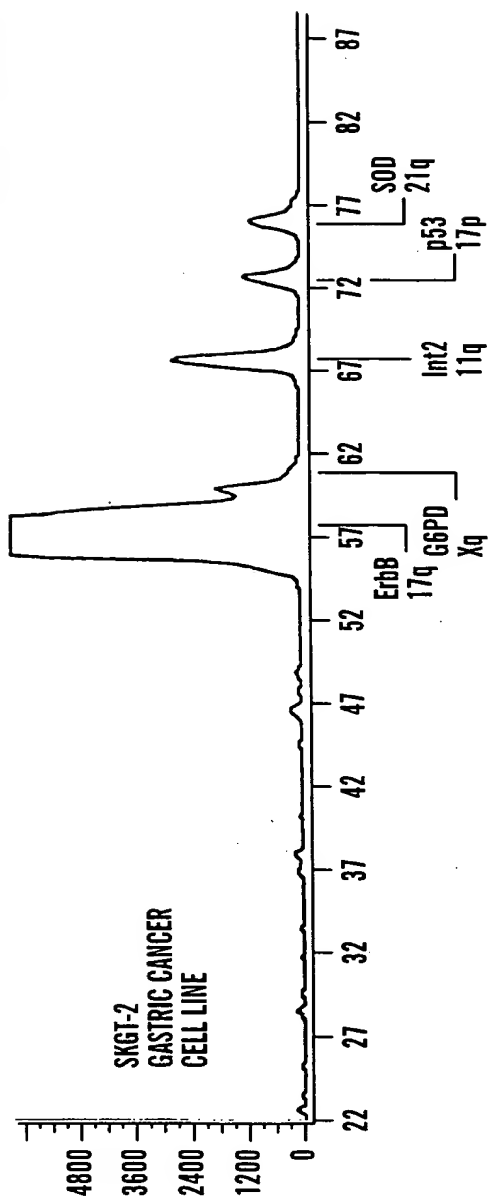


FIG. 27A

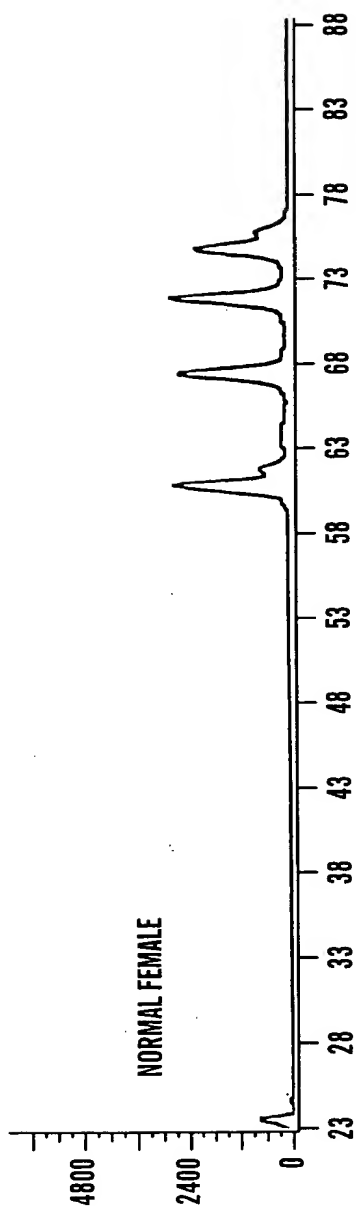


FIG. 27B

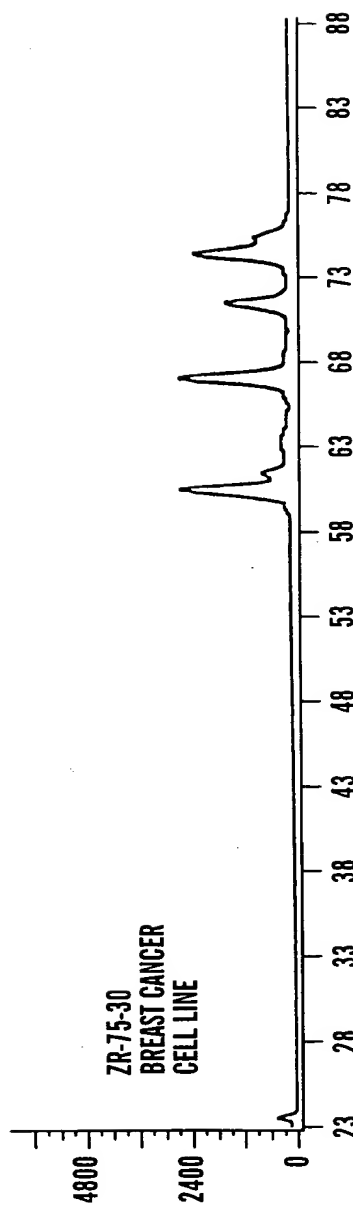
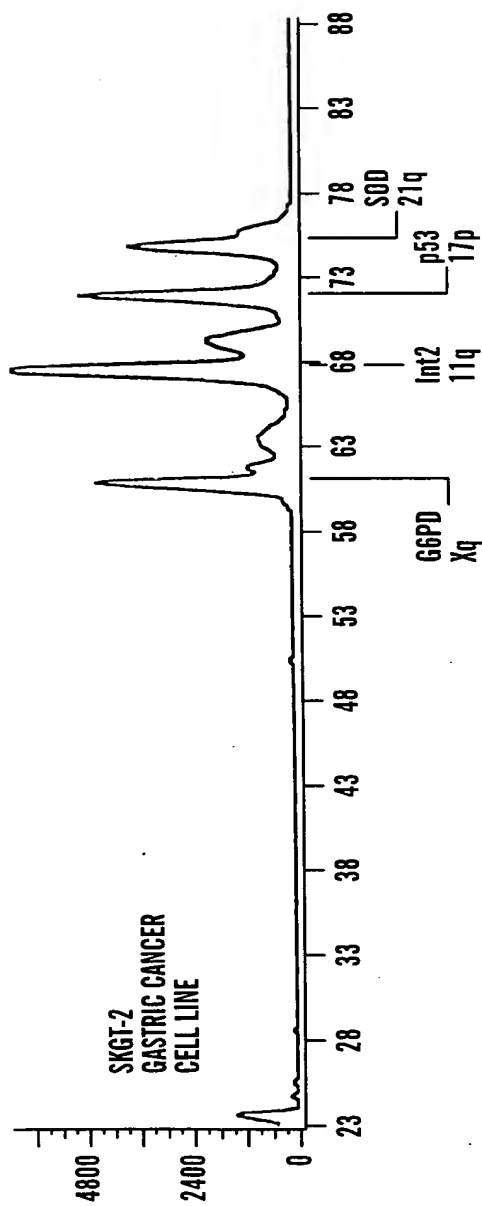


FIG. 27C



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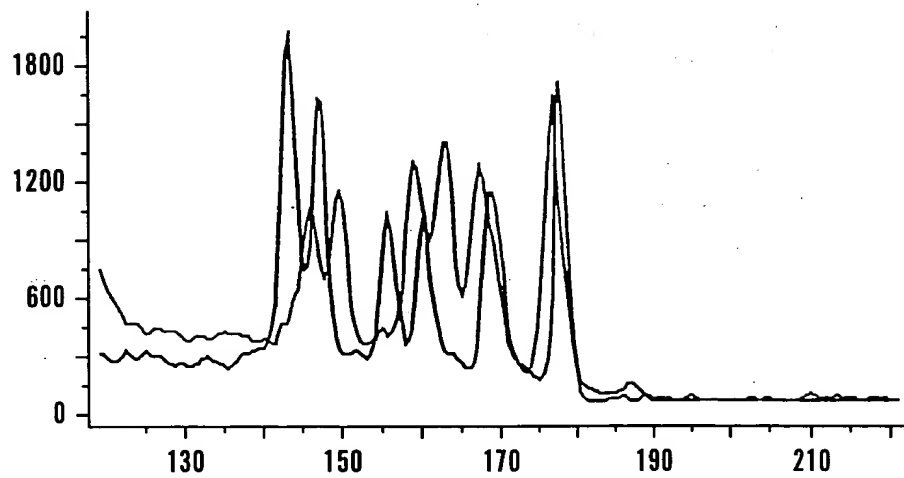


FIG. 28A

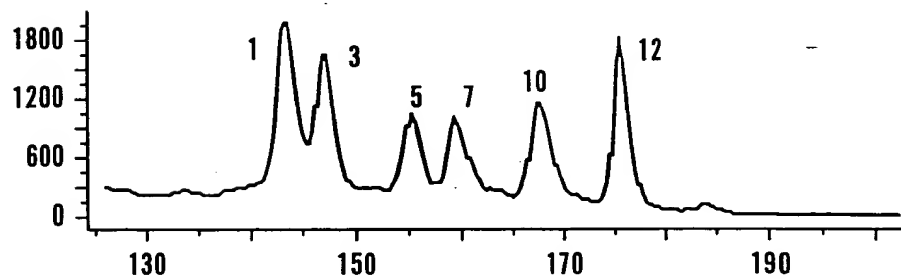


FIG. 28B

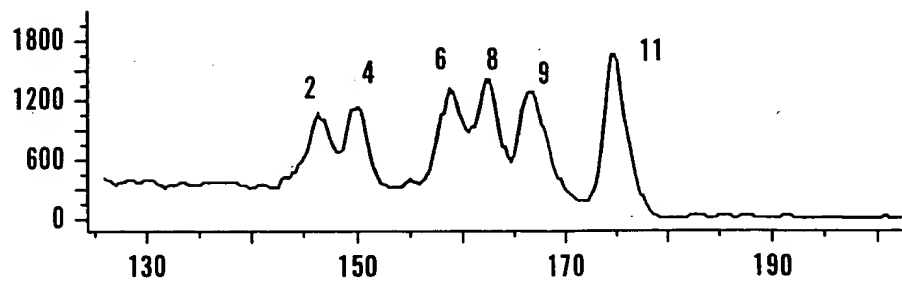


FIG. 28C

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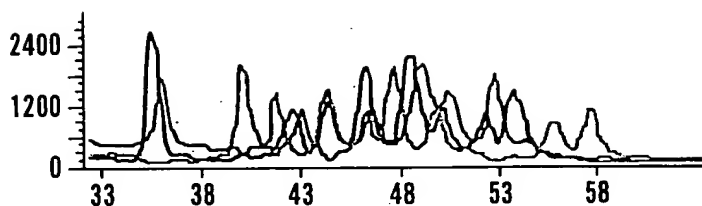


FIG. 29A

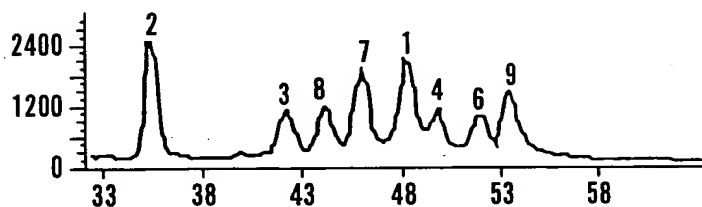


FIG. 29B

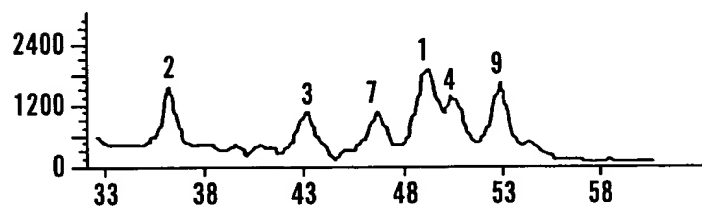


FIG. 29C

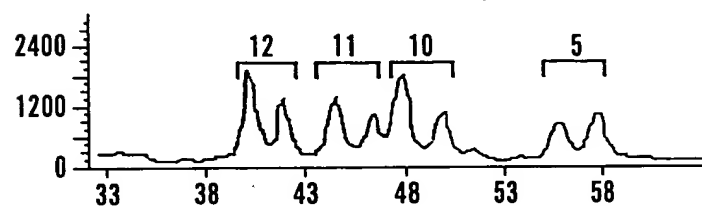


FIG. 29D

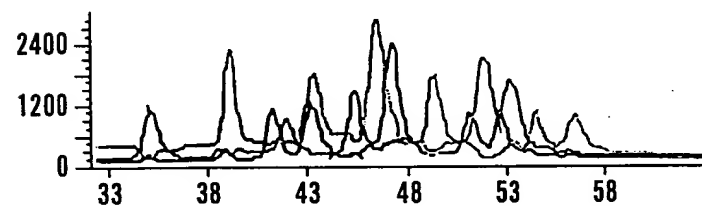


FIG. 29E

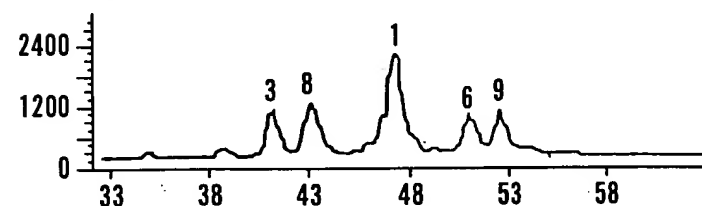


FIG. 29F

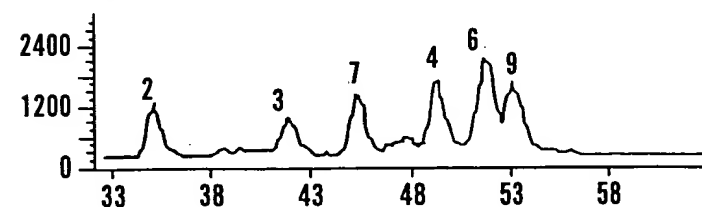


FIG. 29G

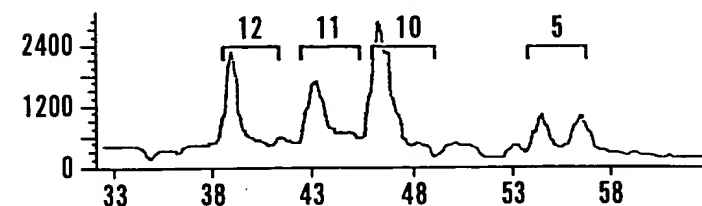


FIG. 29H